Annals of Tropical Medicine & Parasitology

ISSN: 0003-4983 (Print) 1364-8594 (Online) Journal homepage: http://www.tandfonline.com/loi/ypgh19

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To cite this article: J. A. Sinton (1912) Some Observations on the Morphology and Biology of Prowazekia Urinaria (Bodo Urinarius, Hassall), Annals of Tropical Medicine & Parasitology, 6:2, 245-268, DOI: 10.1080/00034983.1912.11687066

To link to this article: http://dx.doi.org/10.1080/00034983.1912.11687066

SOME OBSERVATIONS ON THE MORPHOLOGY AND BIOLOGY OF *PROWAZEKIA URINARIA* (*BODO URINARIUS*, HASSALL)

BY

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(*Received for publication* 30 *May*, 1912)

PLATES XVII, XVIII

CONTENTS

I. INTRODUCTION

The funds for this research, which was carried out in the Liverpool School of Tropical Medicine, were provided by a grant from the Post-graduate Research Fund of the Queen's University, of Belfast. I have much pleasure in thanking Sir Ronald Ross for permission to work in his laboratories and for his kindly interest in the work. Also, I wish to thank Dr. J. W. W. Stephens, Dr. H. B. Fantham, Dr. D. Thomson and Dr. J. G. Thomson for valuable help and suggestions during this investigation.

II. HISTORICAL

The flagellate used in this investigation is a typical member of the genus *Prowazekia* (Hartmann and Chagas, 1910). This genus was separated fom the genus *Bodo* because it was found, by the more exact cytological methods of recent years, that under the term *Bodo* was included two distinct types of flagellates which differed in their nuclear structure and flagellar attachments.

The first reliable account of the cytological details of a member of the genus *Bodo* was the description given by Prowazek (1904) of *Bodo lacertae* (Grassi, 1881). This species does not possess a kinetonucleus. Hartmann and Chagas (1910) described a flagellate *B. cruzi* which, while answering to Stein's definition of the genus *Bodo*, possessed both a principal nucleus and a kinetonucleus. These authors, therefore, thought a new genus should be created to cover this discrepancy, and, as a different nuclear and flagellar apparatus had been described in the only member of this genus (*B. lacertae*) which had been thoroughly worked out, they made a new genus *Prowazekia* to include the Bodo-like flagellates possessing a kinetonulceus, and they named their new species *Prowazekia cruzi*. The name of the new genus was in honour of Prowazek, who, in 1903, figured a structure in *Bodo sp.*, which he called the 'giesselsackchen,' and which was really the kinetonucleus.

This new genus has not been generally accepted yet, as some authorities, notably Alexeieff (1910, 1911*a*, 1911*b*) think that the term *Bodo* should be used as the generic title of the genus which Hartmann and Chagas have called *Prowazekia*, and that the Bodo-like protozoa of the type *B. lacertae*, which does not possess a kinetonucleus, should be included under the genus *Heteromita*, which was the old generic name of *B. lacertae*.

The earliest reference to the occurrence of Bodo-like flagellates in the urine was that of Hassall (1859). This observer found flagellates in 50 samples of urine from a number of different patients, some of whom were suffering from albuminuria and some from cholera. Weakness and debility was a feature of all the cases which showed flagellates in the urine. The reaction of the urine was alkaline or only feebly acid, and the flagellates only appeared after the urine had stood several days. Hassall named

this organism *Bodo urinarius*, and gave a very good description of it. The flagellate measured 14μ by 8μ, and was round or oval in shape, sometimes with one end of the body enlarged. These forms had 'one, usually two, and sometimes three lashes or cilia,' and showed very active motility.

In his drawing of these flagellates, Hassall depicts the round or oval forms, the 'sausage-shaped' forms and the 'carrot-shaped' forms described later on in this article. Cultures were obtained by Hassall on a mixture of an alkaline solution with albumen, and it was noted that the cultural forms were usually smaller than the original flagellates. In 1868, Salisbury* described a similar flagellate in the urine under the name *Trichomonas irregularis*. This parasite was again described by Kunstler (1883) under the name *B. urinarius* (*Cystomonas urinaria*, Blanchard, 1885, *Plagiomonas urinaria*, Braun, 1895). The form which he described was found in a mass of pus in the freshly passed urine of a case of pyelitis. This flagellate was 10 to 15μ long by 4 to 5μ broad, with a broad anterior end and a pointed posterior end. The anterior end showed a depression and a beak-like process from which the flagella originated. This form is similar to the 'carrot-shaped' form described in this article. (Text fig. 3.)

From the descriptions given by Hassall and the above authors I believe the flagellate described in this article to be identical with *Bodo urinarius*, but, because it possesses a kinetonucleus, it must now be placed in the genus *Prowazekia*, and be called *Prowazekia urinaria*. Ultimately, this flagellate may be found to be identical with some of the numerous free-living species of *Prowazekia*.

Recently, several members of the genus *Prowazekia* have been described as occurring in the human faeces, both in diseased and healthy persons in various parts of the tropics. Castellani and Chalmers (1909) described a flagellate *B. asiaticus* in the faeces of a case of ankylostomiasis in Ceylon. This species has been investigated further by Whitmore (1911) under the name *Pr. asiatica*, and they believe it to be a free-living form. A somewhat similar flagellate *Pr. cruzi* was isolated by Hartmann and Chagas (1910) on an agar plate which had been inoculated from

* Quoted by Blanchard, p. 78, 1889.

the faeces of patients in Brazil. (This species was also thought to be a free-living form.) In the same year, Mathis and Leger, in Indo-China, described a form *Pr. weinbergi* in the stools of patients with diarrhoea and also of patients with no intestinal trouble. This species was found to occur in the faeces even when obtained with aseptic precautions, and, therefore, these authors believe that it is an intestinal inhabitant, but with no pathogenic rôle. On account of the ubiquitous distribution in air, dust, water, etc., of the genus *Bodo*, in the old sense, as was pointed out by Alexeieff (1911*b*), I think further evidence is necessary to establish the fact that these flagellates were actually present in the fresh faeces and were not the result of subsequent contamination.

A free-living form *Pr. parva* has been described by Nägler (1910) in the slime on the stones at the biological station at Lunz.

III. OCCURRENCE OF THE FLAGELLATE

The flagellate with which this research was conducted was first observed by me in March, 1912, in the deposit, after centrifugalisation, of a 24-hour old specimen of urine from a Mexican sailor in the Royal Southern Hospital, Liverpool. This patient was suffering from malignant tertian malaria, and had a history of an attack of blackwater fever a few days prior to admission. The same flagellate was again seen in the deposit of a 6-hour old specimen from the same patient a few days later. In the first case, the reaction of the urine was alkaline, and in the second case, neutral. In both cases the urine contained albumen and abundant casts. Neither of the above specimens was taken with aseptic precautions, so the possibility of contamination could not be excluded.

As the old descriptions of the flagellates found in urine were rather vague, and because of the increasing prominence of the genus *Prowazekia* since the discovery in the human faeces of several species, it seemed to me that the flagellate was worth investigating further.

At first it appeared as if the organism had been passed in the urine, and this view was supported by the following facts:—
(1) It had been found on two different occasions in the urine of the

same patient. (2) It could not be found either by direct examination or cultural methods in the urine, taken under the same conditions, of any of the other patients in the same ward, although nearly thirty were examined. (3) Similar examinations of the patients' faeces were negative. (4) The examination of the water supply also gave negative results. (5) Unsuccessful attempts were made to find it in a vessel of water exposed to the air as recommended by Alexeieff (1911*b*) for obtaining *Prowazekia caudata* (*Bodo caudatus*).

These observations are rather negatived by the facts that later specimens taken with aseptic precautions did not show the flagellate, and that a temperature of 37° C rapidly kills the cultures. I am, therefore, inclined to think, from the evidence as a whole, that this flagellate was probably not an inhabitant of the urinary tract, but was an accidental contamination.

IV. TECHNIQUE

(1) *Isolation of 'pure' cultures.*

In examining cultures of protozoa, obtained from the faeces or urine, or in fact any protozoal cultures, it is very important for the investigator to make sure that his culture, which apparently contains only one species, does not contain several closely allied species or members of several closely allied genera. The flagellate used in this investigation was isolated in 'pure' culture by the method recommended by Sellards (1911) for the isolation and culture of amoebae.

A little of the infected material was well diluted with sterile water. Then a number of minute drops of dilute serum (1 in 25 pleural fluid) were placed on a series of sterile cover-glasses. These drops were of such a size that with a 1/2 inch objective the whole drop could be seen at once. A straight platinum wire was then dipped into the diluted culture, and each of the drops was touched with it. These drops were then examined with the 1/2 inch objective until one was found which contained a single flagellate. To this drop a little more dilute serum was carefully added, and the drop was spread out slightly, so that in future examination it would not be too deep for all parts to be seen with a 1/12 inch

objective. This coverslip was then inverted over the cell of a hanging-drop slide and ringed with vaseline, and the drop was again examined very carefully, with both 1/2 inch and 1/6 inch objectives, to make sure that it contained only one flagellate, and also that no cysts were present.

It may be noted here that in the early stages of multiplication the daughter forms produced by the first one or two divisions tend to remain near the spot where the parent cell was. This fact is of importance in the examination of these hanging-drop cultures, because, if two collections of six or eight cells are found at opposite sides of the drop, while the intermediate space is free from cells, it is almost certain that these collections have originated from separate parent cells and that, therefore, the resulting culture may not be 'pure.' By this method it is not only possible to obtain a culture from a single flagellate, but at the same time the rate of multiplication and the mode of formation of division rosettes may be noted.

Ordinary cultures may be obtained from these hanging-drop cultures by removing the coverslip, and inoculating suitable media from it with a platinum loop.

(2) *Examination of Fresh Preparations.*

In studying the biology of this flagellate, fresh cover-glass preparations were found to be more useful than hanging-drop preparations. In these cover-glass preparations the very active motility of the organisms had usually slowed down sufficiently in 1/2 to 1 hour to permit the flagellar movements, the ingestion of food, etc., to be observed. A truer idea of the characters of the motility could be observed in hanging drops.

(3) *Examination of Fixed Preparations.*

Another method of making preparations is to place a drop of the culture on a slide and expose it to the vapour of osmic acid (4 %) for fifteen to thirty seconds, and then place a cover-glass on this drop. By this method the morphology can be carefully studied, and measurements can be made more accurately than in stained preparations, because there is not the same liability to shrinkage and distortion as so often occurs in stained smears. The

examination of these preparations is greatly facilitated by adding a little dilute haematoxylin solution to the drop before covering it. This method shows up the two nuclei very well, and in some cases even the origin of the flagella from the basal granules can be made out.

(4) *Staining.*

The stains used were iron-haematoxylin, glycerine haematin, Delafield's haematoxylin, Giemsa's stain, Leishman's stain, and Romanowsky's stain. Heidenhain's original iron-haematoxylin method was found to give better results than Rosenbusch's modification of this method. In both cases it was found that the flagellates on films fixed with osmic acid vapour for thirty to forty-five seconds kept their natural shape better than those fixed in hot sublimate alcohol (Schaudinn's fluid).

To obtain good results with Giemsa's or Leishman's stain the following method of preparation and fixation of the films was found to be the best.

(1) A drop of fresh serum was mixed on a slide with a drop of the culture.

(2) This drop was fixed in osmic acid vapour for thirty to forty-five seconds and a film was then made of it.

(3) This smear was again exposed to osmic acid vapour for the same time and it was then ready for fixation and staining. In staining with Giemsa stain the film, made as above, was

(4) Fixed in methyl alcohol twenty minutes.

(5) Stained with dilute Giemsa's stain (one drop to 1 c.cm. of distilled water) for one to three hours.

(6) Wash off the stain with tap water. Place a cover-glass on the wet film and examine with the 1/6 inch objective, and if the staining is too deep, repeat the washing until the correct colour is obtained. A rapid rinse in orange-G-tannin solution will greatly shorten this process in deeply stained films, but the results are more uncertain.

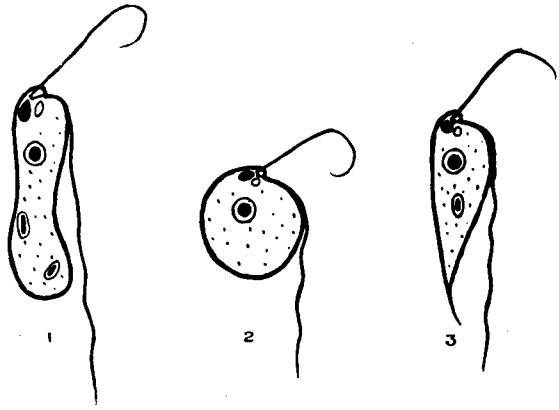
(7) Blot almost dry, clear in xylol, and mount in acid-free Canada balsam.

The films may be stained with Leishman's stain in the ordinary way, but it is better to allow the diluted solution to act on the

film for at least thirty minutes, and to regulate the amount of washing afterwards by frequent microscopic examinations. It was found that the Giemsa method showed up the flagellar attachments better than the Leishman method did, whereas the latter demonstrated the nuclear karyosome better. (Plate XVIII, figs. 12 and 14.)

V. MORPHOLOGY

This protozoon is a typical specimen of the genus *Prowazekia* (Hartmann and Chagas, 1910), formerly included in the genus *Bodo*. It possesses two flagella (an anterior and a lateral), and two nuclei (a principal nucleus and a kinetonucleus or blepharoplast). Morphologically, it bears a very close resemblance to some members of the genus *Trypanoplasma*, especially those with cytophagous habits, and appears to differ from these forms chiefly in the fact that the lateral flagellum is not joined to the body by an undulating membrane.



TEXT-FIGS. 1-3.—Types of *Prowazekia urinaria*
1.—'Sausage-shaped' form
2.—Round form
3.—'Carrot-shaped form

For the purpose of description in this article, the side of the body along which the lateral flagellum lies will be termed the flagellar side, while the opposite side will be called the aflagellar side.

(1) *Shape and Size.*—In cultures, three types are found (*a*) the

sausage-shaped' type; (*b*) the round or oval type; and (*c*) the 'carrot-shaped' type.

(*a*) The 'sausage-shaped' type (Text fig. 1, Plate XVII, figs. 5-8) is long and cylindrical with rounded ends which are usually somewhat thicker than the rest of the body. This type is often slightly curved, with its concavity towards the flagellar side. Its average length is about 18μ by 4μ, but forms varying from 10μ by 2·5μ to 25μ by 6μ may be seen.

(*b*) The round or oval type (Text fig. 2, Plate XVII, figs. 1-4) varies in size from small round forms 4μ in diameter to large oval forms 15μ by 10μ, with sizes varying between these limits. These forms occur most commonly in old cultures and on the surface of solid media.

(*c*) The 'carrot-shaped' type. These forms (Text fig. 3, Plate XVII, fig. 11) are rounded anteriorly, and taper away to a point posteriorly, the aflagellar side of the body being usually flattened. In transverse section this form is not circular like the two previous types, but is flattened. It shows great variations in size, from small forms 6μ by 3μ to large forms 25μ by 4μ. The external morphology of this form closely resembles *Bodo urinarius*, Kunstler (1883).

At first, it might be thought that these three forms represented different species, but this was disproved by the fact that the 'sausage-shaped' forms upon division may give rise to two daughter cells, one round and the other 'carrot-shaped.' (Text fig. 9.) This was, later, confirmed by isolating a single protozoon, and growing it in a hanging-drop culture after the manner recommended by Sellards (1911). In this culture all three types were found to develop from a single flagellate. In all these forms the aflagellar side is carried forward to form a rounded, beak-like projection or rostrum, which overhangs a **V**-shaped depression on the anterior edge—the cytostome.

(2) *Protoplasm.* When examined in fresh preparations, the protoplasm is seen to contain a large number of small, highly refractile granules, which are more numerous towards the posterior end. The rostrum, being free from granules, appears homogeneous. In most cases, but more especially in specimens taken from cultures on solid media, numerous vacuoles may be seen containing bacteria.

The body is enclosed by a thin periplast, which permits of amoeboid movements of the protoplasm, although no true pseudopodial processes are developed except in dividing forms. When the parasite is leaving a cyst or squeezing through a narrow opening between two masses of bacteria, the body may become distorted, and false pseudopodia may be formed (Text fig. 16, Plate XVIII, fig. 27), which must not be confused with true pseudopodia, because these false pseudopodia are merely the results of pressure, and are not spontaneous outgrowths. These false pseudopodia disappear quickly when the parasite becomes freed. (Text fig. 17.)

In preparations stained with Giemsa's stain the protoplasm appears of a blue colour, the rostrum staining a lighter colour than the rest of the body, while vacuoles containing bacteria in various stages of digestion may be seen, especially towards the posterior end (figs. 5-7 and 16). Sometimes a few rather small vacuoles may also be seen which do not contain any remains of digested matter (figs. 6 and 15) and some of the flagellates may show a few fine red-staining granules scattered through the protoplasm (figs. 1-3, 11 and 12). Most of the flagellates have a well-marked contractile vacuole when examined in fresh preparations, but this cannot be seen in stained specimens. It is situated very close to the base of the cytostome on its aflagellar side, and does not show any iodophile reaction. (Text figs. 1-3.)

(3) *Principal nucleus* (*Trophonucleus*). In the elongated forms it is situated in the anterior half of the body, and is usually eccentrically placed, being nearer the aflagellar margin. When seen in fresh preparations the nucleus is round or oval in shape with a well-marked karyosome, which has a clear area around it, bounded by a distinct nuclear membrane. The size of the nucleus does not vary much in the trophic forms, being usually 2·5μ to 3·5μ in diameter, while the karyosome is about 1·5μ to 2μ.

The karyosome is well seen in specimens stained with Leishman's stain. It takes up a bluish colour while the extra-karyosomal chromatin shows up as fine reddish granules (figs. 12 and 14). In Giemsa preparations the nucleus is reddish purple in colour, and appears granular, and in most cases it is usually impossible to distinguish the karyosome clearly.

Using Heidenhain's iron-haematoxylin stain, the karyosome stains black with a clear area around it, and it usually takes on lighter colour with this stain than the kinetonucleus does. If a drop of fresh material was fixed in osmic acid vapour and mixed with a little dilute haematoxylin stain, it was found that the karyosome took up the stain much more rapidly than the kinetonucleus.

(4) *The kinetonucleus* or *blepharoplast* is situated close to the base of the rostrum, on the aflagellar side of the body, usually being in contact with the periplast. In fresh preparations it may not be so easily seen as the principal nucleus. It is a relatively large body, usually pear-shaped with the apex pointing forward (fig. 15), but sometimes, especially in forms about to divide, it is larger and more distinct, and is round or oval in shape (fig. 16).

In specimens stained with any of the Romanowsky stains it appears as a homogeneous body of a deep reddish purple colour, and is always darker than the principal nucleus. The kinetonucleus measures 2μ to $3{\cdot}5\mu$ in its greatest diameter. The relatively large kinetonucleus of this protozoon resembles very closely the kinetonucleus or blepharoplast of some Trypanoplasms.

(5) *The Flagella* are two in number, an anterior and a lateral. The anterior flagellum, which is the shorter of the two, is directed forwards and is thicker and more motile than the lateral flagellum, being always in continuous motion, coiling and uncoiling rapidly. This motion chiefly affects the anterior two-thirds of the flagellum, the basal portion being more rigid, and the coiling movements are always towards the flagellar side. The lateral flagellum, which is usually twice as long as the anterior one, is directed backwards and lies in close apposition with the body for the first part of its course, the remainder trailing freely behind the organism. It is thinner and more wavy than the other flagellum. The anterior flagellum measures from 8μ to 12μ in length, while the lateral varies from 15μ to 25μ. There is no constant relation between the size of the body and the length of the flagella, but the flagella are usually longer in the smaller forms in proportion to the size of the body (figs. 1-3 and 8.)

The third flagellum described by Hassall in some individuals was probably the long, thin, pointed end of the 'carrot-shaped' forms. (Text fig. 3.)

In well-stained Giemsa preparations the flagella stain red, and can be seen to arise from two small red-staining bodies, the basal granules ('diplosome' of Prowazek); these are often so close together as to appear as a single body. They are situated very near the apex of the kinetonucleus, and in some cases appear to be actually in contact with it (fig. 6). A very fine thread joining these granules to the kinetonucleus has been observed once or twice in well-stained specimens (fig. 17). This thread probably corresponds to the 'rhizoplast' observed by Hartmann and Chagas (1910) in *Pr. cruzi*, and by Whitmore (1911) in *Pr. asiatica*. The anterior flagellum runs directly forward and leaves the body at the apex of the rostrum. The lateral flagellum runs transversely outward along the anterior margin and leaves the body as a free flagellum near the junction of the anterior and lateral margins, at which point it turns directly backwards.

The origin of the flagella and the course taken by them show a very close resemblance to similar structures in *Trypanoplasma ranae* (Walker 1910), which resemblance is still further accentuated by the presence in this trypanoplasm of a slight depression at the anterior end, which Walker suggests may represent a cytostome.

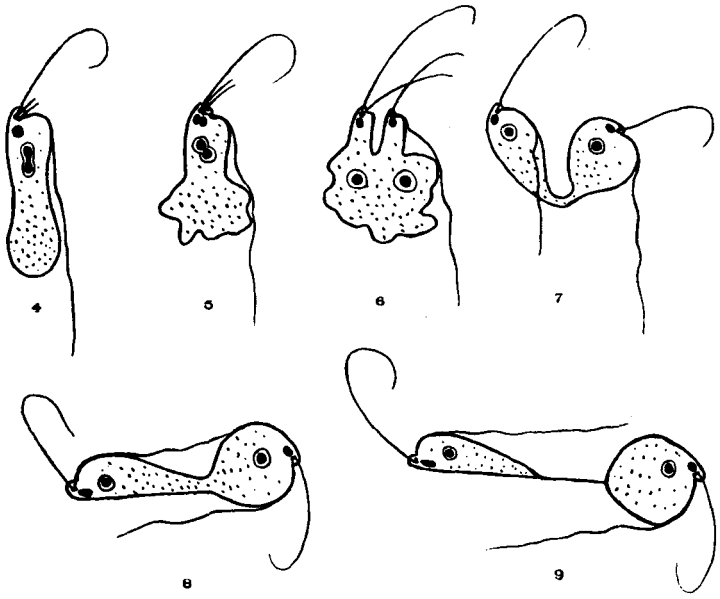
VI. MOVEMENTS

All the trophic forms are very actively motile, the elongated forms being especially so. The movement is of a jerking character and is seen to be produced by the coiling and uncoiling of the anterior flagellum or tractellum, while the lateral flagellum or gubernaculum acts as a rudder. In hanging-drop and fresh preparations the flagellate may be seen to move actively about among the masses of bacteria, and every now and then it attacks one of these masses. While the organism is 'rooting about' among the bacteria it is anchored to the mass by the lateral flagellum. If a flagellate, during its wanderings, gets into a channel between the bacteria which is too small to permit further progress, it must turn around before it can get out, because the organism is only capable of motion with the anterior end forward, thus differing from members of the genus *Trypanosoma*, which can move with either end forwards. This turning movement is rendered possible by the amoeboid character of the protoplasm.

Before division or encystment, the movements become very sluggish, and forms taken from the surface of solid media are not very motile at first, but on being transferred to a fluid medium, very soon become active again.

VII. MULTIPLICATION

By isolating and cultivating a single organism in a hanging-drop preparation by the method of Sellards (1911), it was found that at room temperature this single cell divided into two, in times varying from twelve to twenty-four hours, but that as soon as division had once taken place it occurred again every four hours, so that twenty-four hours later it was possible to count approximately one hundred and twenty-eight cells in the drop, after which time it was impossible to enumerate them.



TEXT-FIGS. 4-9.—Showing method of division

In fresh preparations, if the elongated forms be examined, the first sign of multiplication is the appearance of two new flagella, growing out from the apex of the rostrum. (Text figs. 4-5, Plate XVIII, fig. 20.) These flagella at first are short and thick, but they soon increase in length and become thinner. At the same

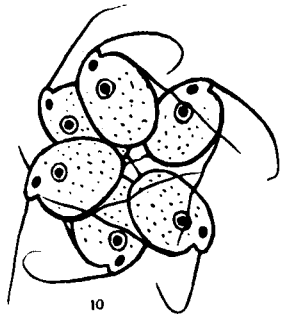
time, the movements of the cell become very sluggish and all the food vacuoles disappear. The posterior end of the body gradually accumulates a large number of small refractile granules, and becomes very amoeboid, even showing true pseudopodia of small size. (Text figs. 5-6.) This amoeboid movement slowly extends forward until the whole body is involved. A split appears at the anterior end between the two new flagella and extends gradually backwards, until we get a form like **Y**. (Text figs. 6-7.) The very amoeboid character of the cell at this stage gives rise to forms, which, if the act of division had not been carefully followed from the start might be wrongly interpreted. Thus, when division has proceeded a little further, the two limbs of the **Y** may straighten out, giving rise to a form which is apparently undergoing transverse division. (Text fig. 8.) At another time when the active movements of the two daughter cells have stretched the connecting protoplasm between them to a narrowed band, if the movements cease, the two cells are drawn together and partly fuse again, an appearance, which, if seen first at this stage might be mistaken for conjugation, especially if the conditions of examination are unfavourable to the cell and complete division does not occur before death takes place. The occurrence of well-marked pseudopodial processes at this stage gives rise to very curious forms.

The two daughter-cells, by their exertions, gradually stretch the connecting link until it becomes a very fine, highly refractile, elastic thread, often 60 or 70μ long (Text fig. 9), this soon breaks, liberating the two cells. Immediately after division, although the movements of their flagella may be active, the movements of the daughter cells are slight, being apparently hampered by the relatively long flagella, but active motility is soon attained. The daughter cells may be equal or unequal in size, and may conform to different morphological types (Text fig. 9). In the division of a rounded form the whole body becomes amoeboid, and a clear line of demarcation appears across it.

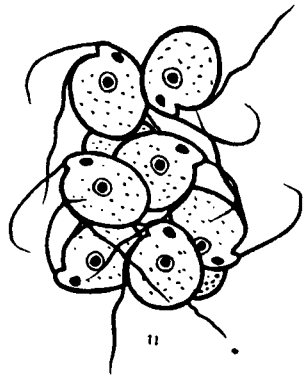
The principal nucleus may divide before the kinetonucleus, but the reverse may occur in other cases. The division of the principal nucleus appears to take place in two ways. In one case the equatorial plate is formed by the karyosomal chromatin, while in the other method it is the extra-karyosomal chromatin which

forms the equatorial plate. The kinetonucleus when dividing becomes elongated, the central portion becomes narrower and narrower, and gradually the kinetonucleus divides into two. The phases of nuclear division, which I have observed, seem to be similiar to those figured by Alexeieff (1911*a*) in *Prowazekia caudata* (*Bodo caudatus*).

In stained preparations the new flagella appear to grow out from the two basal granules (fig. 20), and not to be formed by the splitting of the original flagella. In some cases where division is very active the daughter cells divide again before they are completely separated, giving rise to division rosettes composed of four to eight small individuals, with their anterior flagella pointing outwards. (Text fig. 10.) These should not be confused with the



TEXT-FIG. 10.—Division rosette



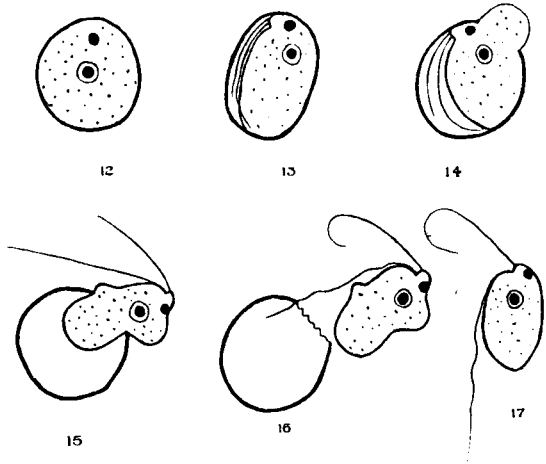
TEXT-FIG. 11.—Mass of agglomerated flagellates

agglomerated masses which are sometimes seen, but which usually contain a larger number of cells, and in which the flagella may point in any direction. (Text fig. 11.) These agglomeration masses seem to be formed by the sticking together of very young forms. These very young forms when they come in contact with other young forms tend to adhere, whereas the older forms do not. It is possible that some of these division rosettes may be formed from the large vacuolated amoeboid masses, about 18μ by 10μ with multiple flagella, which are sometimes observed.

VIII. ENCYSTMENT

In old cultures very numerous round or oval cysts 5μ to 7μ in diameter may be seen. In fresh preparations these cysts are seen to be enclosed by a very thin cyst wall. They are highly refractile bodies with numerous granules inside them, and in some cases a nucleus may be seen (Text fig. 12). Before encystment the cell becomes almost motionless, the movements of the flagella are very sluggish, then, apparently, the thin cyst wall is secreted around the cell, and the flagella are withdrawn inside this cyst.

In good preparations stained with Giemsa the cyst shows a dark blue protoplasm with a nucleus and a kinetonucleus, as well as two folded-up flagella, and the delicate cyst wall can usually be made out, staining a reddish colour (figs. 22 and 23).



Text-Figs. 12-17. Flagellate emerging from cyst

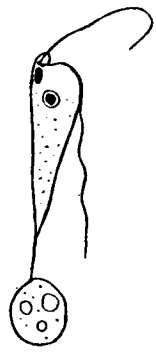
If cysts are placed in a fresh culture medium the enclosed organisms can soon be seen to emerge from them. The first indication of this is that the granules begin to move and the parasite can be seen to be slowly turning round inside the cyst with spasmodic jerks. A little later it is possible to see the two flagella working actively, and at the same time the flagellate makes violent efforts to rupture the thin cyst wall, causing it to bulge in places (Text fig. 13). Finally, the wall bursts and the anterior end of the organism with the two flagella becomes protruded

(Text figs. 14-15, and Plate XVIII, figs. 24-26). After a short quiescent period the flagellate begins to make renewed attempts to free itself, and gradually it emerges, leaving the very thin, almost invisible cyst wall behind (Text fig. 16).

Immediately after leaving the cyst the flagellate may be deformed (Text fig. 16 and Plate XVIII, fig. 27), but its normal shape is soon regained. At first, the movements are sluggish, but this is only for a very short time.

Nägler (1910) and Hartmann and Chagas (1910) describe cysts containing several nuclei in the forms observed by them, but neither in fresh nor in stained specimens have I been able to see any indication of two or more cells being contained in the same cyst.

In examining preparations containing cysts, great care must be taken not to confuse the cysts of various fungi, which may contain a number of spores, with the cysts of the flagellate. Alexeieff (1911*c*) has pointed out that this mistake has probably occurred in



18

Text-Fig. 18.—Separation of vacuolated mass from 'carrot-shaped' flagellate

the investigation of *Trichomonas intestinalis*. Once or twice, a rounded vacuolated mass of protoplasm, 4 to 5μ in diameter, was noticed to separate off from the posterior end of some of the elongated forms (Text fig. 18 and Plate XVII, fig. 9), the exact significance of which was not clear.

IX. BIOLOGY

(1) *Cultivation*. This protozoon was found to multiply freely in urine, forming a scum on the surface, but dying out on the ninth day. Sub-cultures were made on ordinary salt agar, nutrient agar,

serum agar, blood agar, peptone salt solution, nutrient broth, and diluted blood serum. In all these media an abundant growth was found to take place in symbiosis with bacteria. One of the best media was found to be ordinary agar, to the condensation water of which a little blood serum had been added; on this media, motile forms and cysts can be found for at least eight weeks at room temperature (10-20° C). In cultures made on solid media the forms in the water of condensation tend to be elongated, while round and oval forms will be found living among the colonies of bacteria on the surface of the media. On the surface of fluid culture media a scum forms, and upon examination is found to consist of countless numbers of these organisms showing very active motility. This protozoon dies inside twelve hours in anaerobic cultures. Its love of oxygen is also shown by the fact that in fresh cover-glass preparations they tend to congregate around air bubbles or at the edge of the coverslip if it is not ringed with vaseline, and by the fact that in cultures of liquid media made in deep tubes, the protozoon is only present on the surface and not in the depth of the culture.

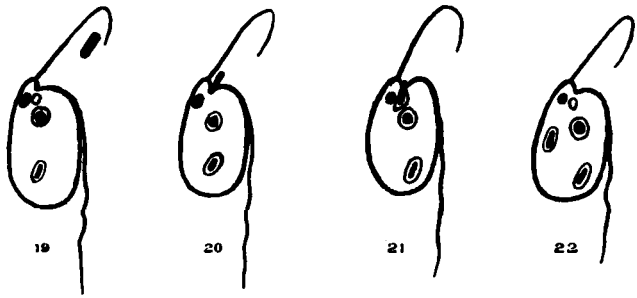
The best temperature for growth seems to be about 20° C, but growth occurs at 30° C. Higher temperatures rapidly kill the cultures, a temperature of 37° C killing them inside one or two hours.

All attempts at growing this flagellate in the absence of bacteria were unsuccessful.

In none of the articles on the cultivation of *Prowazekia* from the faeces have I been able to find any reference to the temperature at which the cultures were grown, nor whether the organism found was aerobic or anaerobic. These points seem to me to be of considerable importance in determining whether these organisms were actually living in the intestine or were merely accidental contaminations after the faeces were passed.

(2) *Capture and Ingestion of Food.* Although the very thin free end of the anterior flagellum, which also acts as a feeler, is capable of grasping bacteria, it is usually by the coiling movements of this flagellum that they are propelled towards the cytostome (Text fig. 19). The food enters the cytostome and, by the aid of well-marked infolding movements of the lips of the cytostome,

is received into a vacuole which forms at the bottom of the cytostome (Text figs. 20-21 and Plate XVII, fig. 1). The vacuole quickly travels towards the posterior end of the body, and in its passage takes a definite course, passing between the principal nucleus and the kinetonucleus along the aflagellar side of the body (Text fig. 22). The cytostome is capable of being greatly distended, as was noticed when flagellates were seen trying to swallow red blood cells, and also when trying to swallow large masses of bacteria.



TEXT-FIGS. 19-22.—Stages in ingestion of bacillus as food

All the bacteria which are propelled towards the cytostome by the anterior flagellum are not ingested, but the organism seems to have a special liking for certain bacteria. This was well illustrated in one case where a small oval flagellate attempted to swallow a strepto-bacillus which was longer than itself, but finding, after most of the bacillus was swallowed, that it was impossible to swallow all of it, the organism disgorged the bacillus again. This same bacillus was immediately attacked by another organism with a similar result, and the same performance was gone through by at least twelve separate protozoa, all of which made unsuccessful attempts to swallow it. Sometimes two flagellates started at opposite ends of this bacillus at the same time and tried to swallow it. This preference for special bacteria is also shown by the way these flagellates in fresh preparations tend to accumulate around certain clumps of bacteria, while other clumps are untouched.

In stained preparations the ingested bacteria are seen to be contained in vacuoles, and to be in various stages of digestion.

(3) *Excretion.* When living specimens are examined, the food vacuoles are seen to accumulate at the posterior end of the body.

After a time, these vacuoles approach the surface of this part and burst, discharging any undigested contents. No distinct cytopyge could be made out.

The contractile vacuole is best seen in the larger flagellates, and measures about 1μ to 3μ in diameter when in diastole. It is situated very close to the base of the cytostome, and is intimately connected with it, seeming to be joined to it by a short canal. The formation of this vacuole is usually rapid, and contraction takes place every fifteen to thirty seconds at a temperature of about 20° C, but at lower temperatures and under adverse conditions it may only occur every two to three minutes.

It was thought at first, from its intimate connection with the cytostome, and because it disappeared when a food vacuole was formed, that the contractile vacuole was really the distended end of the cytostome, but later it was found that, when material containing living flagellates was mixed with some Indian ink, none of the particles appear in the vacuole. From these observations it seems improbable that the contractile vacuole is formed by the dilatation of the fundus of the cytostome.

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EXPLANATION OF PLATES XVII, XVIII.

The figures were drawn with an Abbé camera lucida, using ocular No. 4 and a Leitz 1/12 inch oil-immersion objective. All the figures were stained with Giemsa, except fig. 12 and fig. 14, which were stained with Leishman. Magnification 1,650.

PLATE XVII.

Fig. 1. Round form with bacillus in process of being ingested.

Figs. 2-3. Round forms.

Fig. 4. Oval form.

Figs. 5-8. 'Sausage-shaped' forms, with numerous ingested bacteria.

Figs. 9-10. Intermediate forms between the 'sausage-shaped' forms and the 'carrot-shaped' forms.

Fig. 11. 'Carrot-shaped' form.

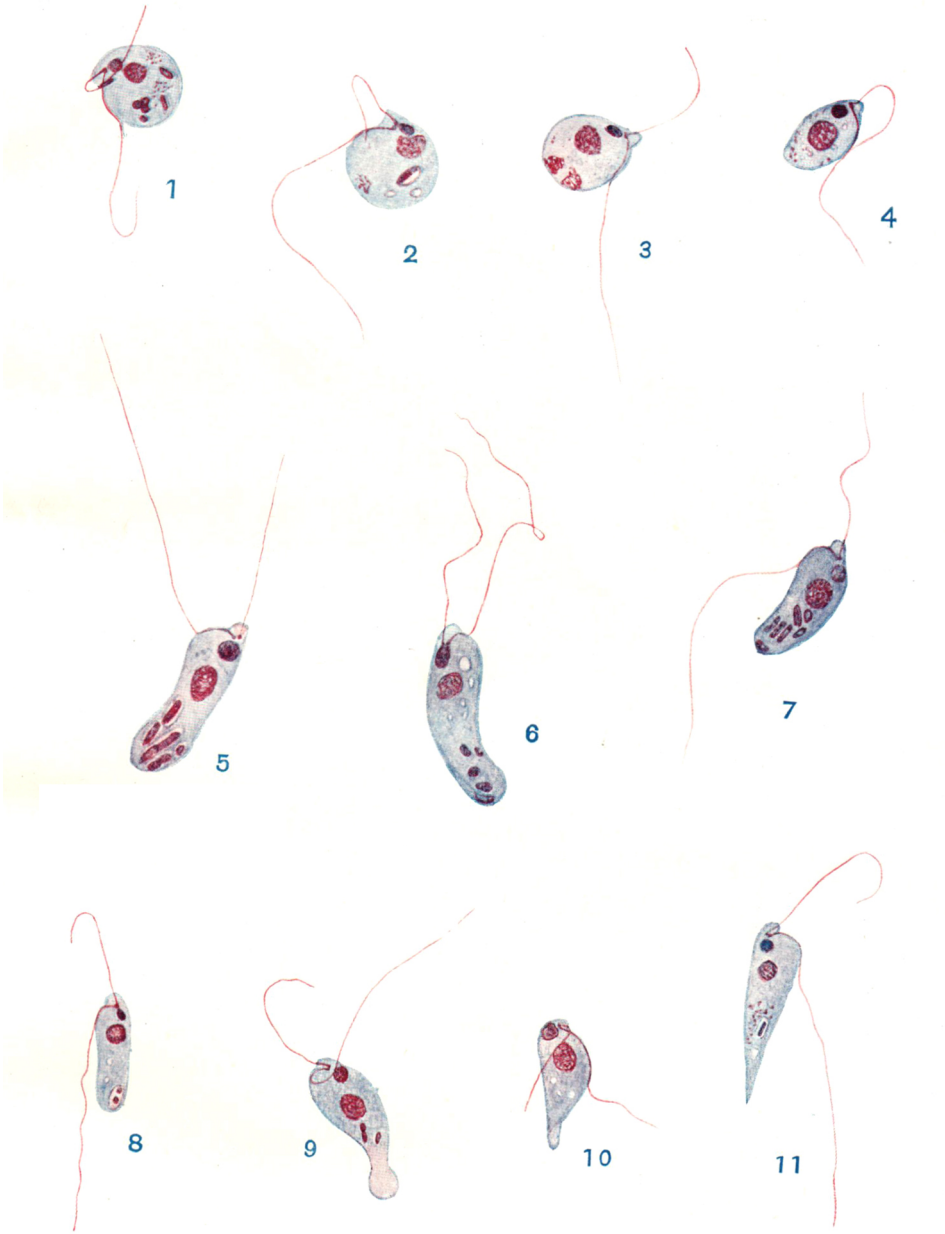

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PLATE XVIII.

Figs. 12-14. Show the nuclear karyosome and extra-karyosomal chromatin.

Fig. 15. Round form with a large pear-shaped kinetonucleus.

Fig. 16. 'Sausage-shaped' form with a very large round kinetonucleus.

Fig. 17. Shows the 'rhizoplast' connecting the basal granules with the kinetonucleus.

Figs. 18-19. Forms with dividing nuclei, each showing two karyosomes.

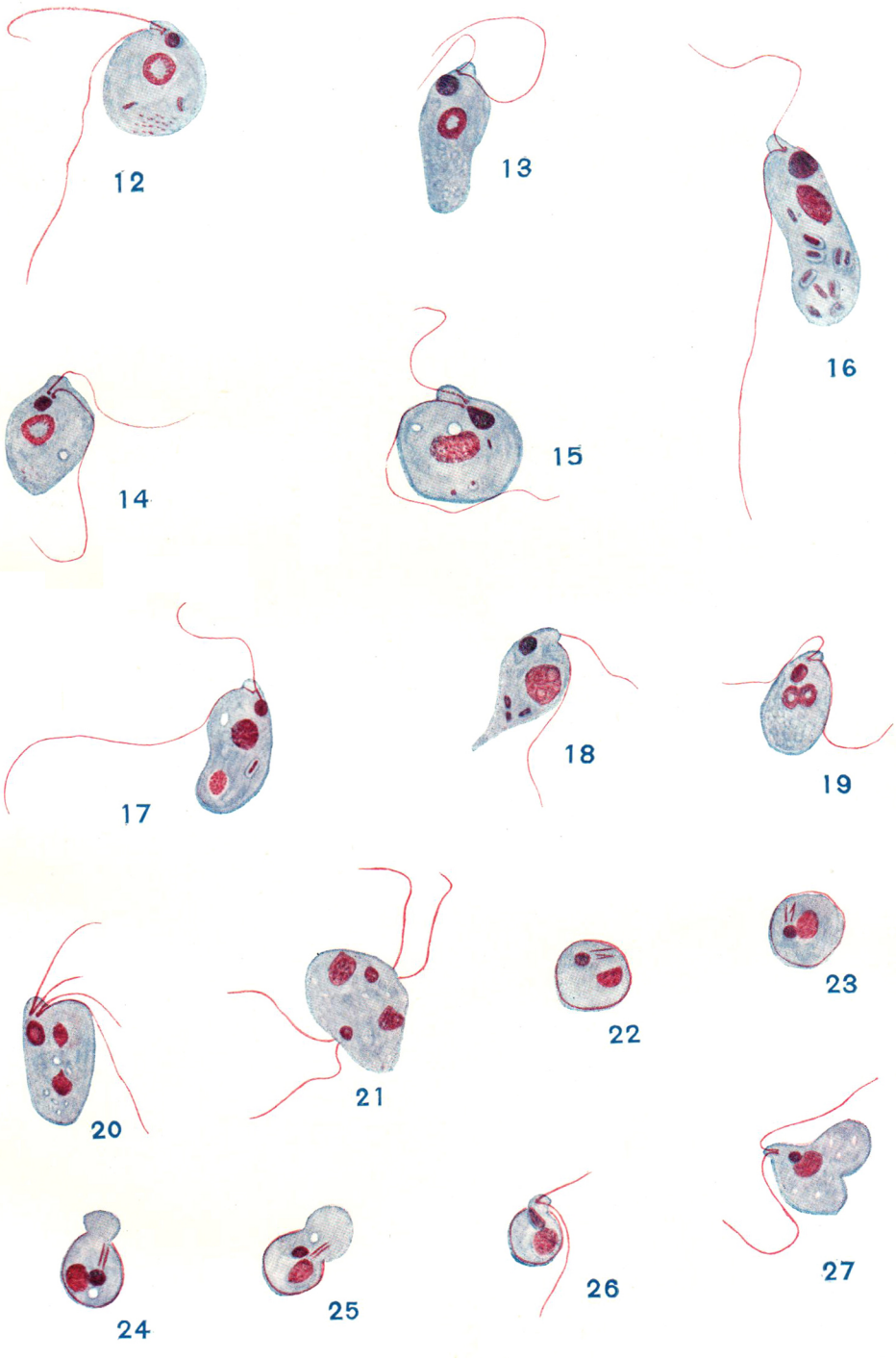
Fig. 20. In this form the principal nucleus has divided, and two new flagella have grown out from the basal granules.

Fig. 21. Amoeboid dividing form, with two principal nuclei and two kinetonuclei.

Figs. 22-23. Cysts showing folded-up flagella.

Figs. 24-26. Flagellate emerging from cyst.

Fig. 27. Flagellate immediately after leaving a cyst, showing a 'false pseudopodium.'



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